Proc. Nat. Acad. Sci. USA
Vol. 72, No. 11, pp. 4655–4659, November 1975
Physiology

Dopamine receptor binding in the corpus striatum of mammalian brain

(phenothiazine/butyrophenone/catecholamine/schizophrenia/adenylate cyclase)

DAVID R. BURT, S. J. ENNA, IAN CREESE, AND SOLOMON H. SNYDER*

Departments of Pharmacology and Experimental Therapeutics and Psychiatry and the Behavioral Sciences, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205

Communicated by Julius Axelrod, August 20, 1975

ABSTRACT **Specific binding of [^{3}H]dopamine to membranes from the corpus striatum of rat and calf brain appears to involve the postsynaptic dopamine receptor. Specific [^{3}H]dopamine binding is saturable, with half-maximal binding in calf membranes at 7 nM. Apomorphine is about twice as potent as dopamine in competing for binding sites, whereas (−)-norepinephrine is 5% as potent as dopamine and isoproterenol is virtually inactive. The relative potencies of phenothiazines as inhibitors of specific dopamine binding correlates with their clinical potencies and actions on the dopamine-sensitive adenylate cyclase.**

Effects on dopamine receptors determine actions of major psychotropic drugs (1). Antagonism by neuroleptic "antischizophrenic" drugs of a dopamine-sensitive adenylate cyclase correlates with pharmacological potency, suggesting that the cyclase is associated with the dopamine receptor (2–5). We now report direct binding of dopamine to apparent postsynaptic receptor sites in membrane fractions of the corpus striatum of calf and rat.

METHODS

Caudate or other regions of calf brains, fresh from a slaughterhouse, were frozen at −20° until assayed (up to 2 weeks). Tissue was homogenized with a Brinkmann Polytron PT-10 in 40 volumes of ice-cold 50 mM Tris·HCl buffer, pH 7.7 at 25°. The homogenate was centrifuged at 50,000 × *g* for 10 min on a Sorvall RC-2B centrifuge. The supernatant fluid was discarded; the pellet was rehomogenized in 50 volumes of the same buffer and recentrifuged. The supernatant fluid was discarded, and the pellet was homogenized in 100 volumes of 50 mM Tris·HCl buffer, pH 7.5 at 25°, with 10 μM pargyline and 0.1% (weight/volume) ascorbic acid. This final tissue resuspension was placed in a 37° bath for 5 min and returned to ice for use in the binding assay.

The two preliminary washes with buffer remove endogenous dopamine, while preincubation at 37° ensures inactivation of tissue monoamine oxidase by pargyline before addition of radioactive dopamine.

Male Sprague-Dawley rats were decapitated and the corpus striatum was prepared as described for frozen calf tissue.

[^{3}H]Dopamine (3,4-dihydroxyphenyl[*ethyl-1-*^{3}H(N)]ethylamine, specific activity 8.0 and 8.4 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass., and stored under nitrogen at 4°.

For the standard binding assay, 1-ml aliquots of tissue suspension (10 mg original wet weight) were added to test tubes with [^{3}H]dopamine (final concentration 5 or 10 nM). Most tubes also contained nonradioactive dopamine or other compounds. All solutions were made up fresh in 0.1% ascorbic acid and were added as 50 μl or less. Three 250-μl aliquots of each 1-ml incubation mixture were then removed to plastic microfuge tubes (Beckman, 0.4 ml capacity), incubated at 37° for 5 min, and centrifuged for 3 min on a Beckman Microfuge B. The supernatant fluid was removed by aspiration and each pellet rinsed once with 200 μl of ice-cold incubation buffer. The bottom of each microfuge tube was cut off into a scintillation vial; the pellets were dissolved in 1 ml of Protosol (New England Nuclear Corp.) and radioactivity was determined in 10 ml of Econofluor (New England Nuclear Corp.) or LSC (Yorktown Research) pre-mixed scintillation cocktail on Packard Tri-Carb Liquid Scintillation counters (model 3375 and 3385) at an efficiency of 45–49% for 5 min.

Under these conditions, bound radioactivity in the pellets amounted to 4% or less of that in the incubation mixture. Saturable or specific binding of [^{3}H]dopamine was defined as that not removed by adding a large excess of nonradioac-

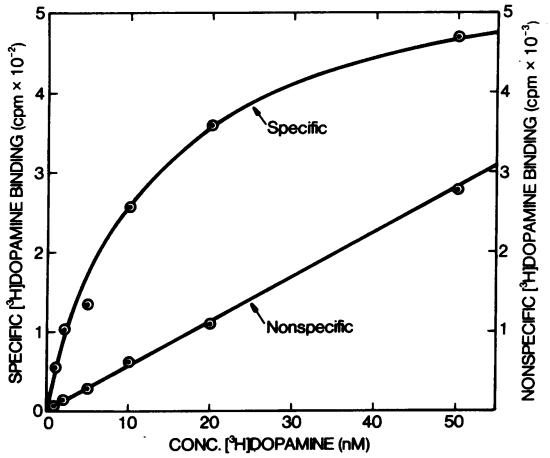

FIG. 1. Saturation of specific [^{3}H]dopamine binding. Increasing concentrations of [^{3}H]dopamine were incubated with membranes of calf corpus striatum equivalent to 10 mg of tissue per ml in the presence and absence of 10^{-6} M (+)-butaclamol. The values observed in the presence of the competitor are labeled "nonspecific." This nonspecific binding was subtracted from the binding observed in the absence of competitors to obtain the binding labeled "specific." Note that the scale for specific binding is expanded 10-fold over that for nonspecific binding. Since the proportion of the total binding represented by the specific binding becomes so low at the higher dopamine concentrations, the possible error in the higher specific binding values is appreciable. Thus, although the triplicate differences between experimental and blank values agreed to within 20% (SD) at 5 nM [^{3}H]dopamine, they agreed only to within 40% (SD) at 50 nM [^{3}H]dopamine.

Abbreviation: IC_{50}, concentration required to inhibit binding by 50%.

* Address reprint requests to this author.

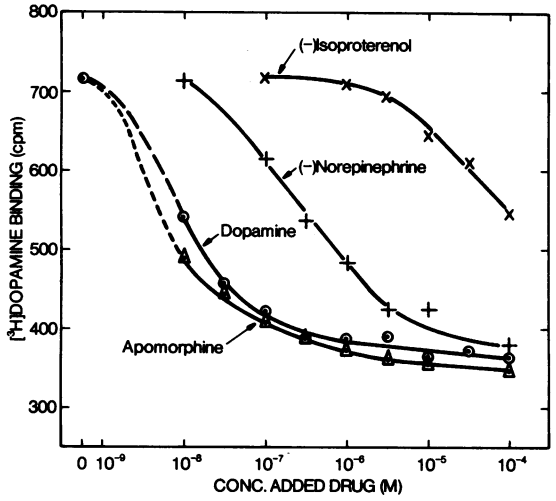

FIG. 2. Competition of catecholamines for [³H]dopamine binding sites. Increasing concentrations of nonradioactive dopamine and other drugs (norepinephrine) were added to tubes containing approximately 5 nM [³H]dopamine and calf striatal membranes equivalent to 10 mg of tissue wet weight per ml. The binding is the mean of that observed in triplicate 0.25-ml aliquots of incubation mixture. Samples were incubated for 5 min at 37° and centrifuged to isolate bound [³H]dopamine. Note that the ordinate on this and succeeding graphs does not start at zero.

tive dopamine or appropriate other drugs to the incubation. Blank values, routinely taken in the presence of 10^{-7} M apomorphine or 10^{-6} M or 10^{-5} M (+)-butaclamol, were subtracted from the total binding to obtain specific binding and were similar with apomorphine and butaclamol. Thin-layer chromatography of membrane-bound tritium revealed a single peak corresponding to authentic dopamine.

In some experiments, separation of bound radioactivity by conventional centrifugation and by filtration (Whatman GF/B Filters) yielded comparable results. The microfuge method was adopted to economize on tissue.

Unilateral substantia nigra lesions were performed as described (6). Three weeks after surgery the rats were tested with 1.5 mg/kg of *d*-amphetamine and demonstrated ipsilateral rotation to the left, indicating a successful lesion of the left nigro-striatal pathway (7).

Apomorphine was from Merck and Co.; nonradioactive dopamine was purchased from Sigma Chemical Co., St. Louis, Mo.; *dextro-* and *levo*-butaclamol were the generous

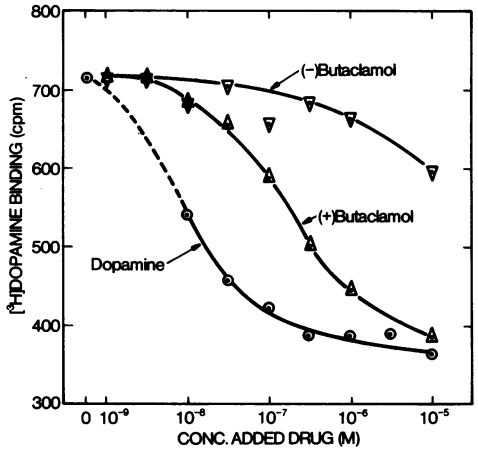

FIG. 3. Competition of stereoisomers of butaclamol for [³H]dopamine binding sites. Data are from the same experiment as Fig. 2.

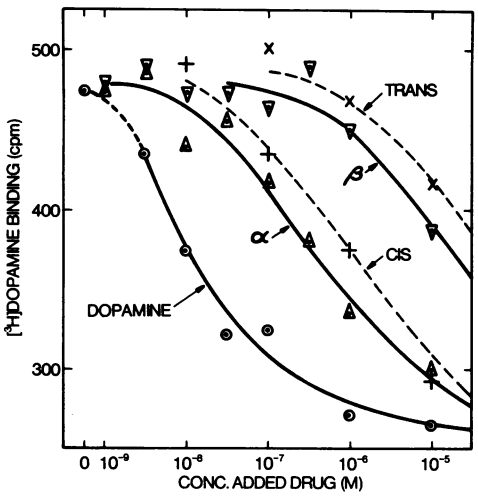

FIG. 4. Competition of α and β isomers of flupenthixol and *cis* and *trans* isomers of thiothixene for [³H]dopamine binding sites. Conditions as in Fig. 2.

gifts of Ayerst Laboratories; α- and β-flupenthixol and *cis*- and *trans*-thiothixene were provided by Dr. P. Seeman; 2-amino-6,7-dihydroxy-(1,2,3,4)-tetrahydronaphthalene was donated by Dr. L. L. Iversen. Haloperidol, droperidol, and benperidol were from McNeil Laboratories. Other chemicals were from commercially available sources or pharmaceutical houses.

RESULTS

General Properties of Specific Dopamine Binding. Specific [³H]dopamine binding to calf striatal membranes is saturable, attaining half-maximal levels at about 7 nM, in contrast to nonspecific binding which increases linearly between 1 and 50 nM (Fig. 1). Scatchard analyses of comparable competition curves with nonradioactive dopamine (Figs. 2, 3, and 5) likewise yield apparent values in the range of 5–10 nM. The number of binding sites in calf striatum corresponds to about 5–10 pmol/g of tissue.

Specific dopamine binding is linear in both calf and rat membranes between 2.5 and 10 mg original wet weight of tissue per ml. Specific binding is optimal at pH 6.9–7.1, with a sharp decline below pH 6.5 using Tris buffers. Time-course experiments indicate that binding reaches equilibri-

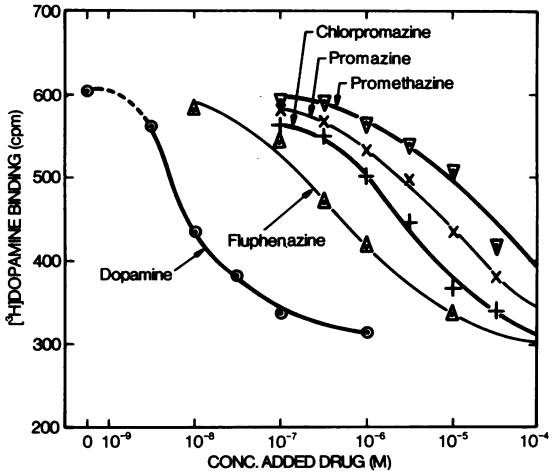

FIG. 5. Competition of phenothiazines for [³H]dopamine binding sites. Conditions as in Fig. 2.

Table 1. Displacement of [^{3}H]dopamine from calf striatal membranes

Drug	IC_{50} (nM)
Apomorphine	4
Dopamine	8
2-Amino-6,7-dihydroxy-(1,2,3,4)-tetrahydronaphthalene	10
Epinine	35
(+)-Butaclamol	125
(−)-Norepinephrine	170
α-Flupenthixol	240
Fluphenazine	325
(−)-Epinephrine	350
Triflupromazine	700
cis-Thiothixene	750
(+)-Norepinephrine	800
Trifluoperazine	825
Spiroperidol	900
Haloperidol	900
Perphenazine	1,200
Bulbocapnine	1,500
(+)-Epinephrine	1,500
Chlorpromazine	1,500
Droperidol	1,800
Clozapine	2,300
Pimozide	2,500
Benperidol	4,000
Promazine	5,500
Hydroxyzine	10,000
β-Flupenthixol	10,000
p-Tyramine	10,000
Promethazine	15,000
(−)-Butaclamol	17,000
Benztropine	19,000
trans-Thiothixene	22,000
Imipramine	25,000
Isoproterenol	57,000

Other drugs with IC_{50}s greater than 10,000 nM: (−)-amphetamine, (+)-amphetamine, cocaine, harmaline, α-methyldopa, α-methyl-*p*-tyrosine, diphenylhydantoin, ketamine, acetophenetidine, chlormezanone, lidocaine, ethosuximide, propranolol, methadone, naloxone, morphine, scopolamine, atropine, reserpine, γ-aminobutyric acid, amatadine, methylphenidate, probenecid, octopamine, serotonin, glycine, carbachol, pyridoxine, isoniazid, tetracycline, aminosalicylic acid, nicotinamide, pyridoxal, α-naphylthiourea, urea, hippuric acid, melatonin, alloxan, panthothenic acid, oxotremorine, physostigmine, benactyzine, meprobamate, and pentobarbital. Data shown are the concentration of each drug required to inhibit specific binding of 5 nM [^{3}H]dopamine by 50% in experiments such as those illustrated in Figs. 2–5. Blanks to determine specific binding were taken as the mean of binding observed in the presence of 10^{-6} M (+)-butaclamol and that in the presence of 10^{-7} M apomorphine or dopamine. Each value is the mean of at least two determinations utilizing four concentrations of drug in triplicate. Such determinations varied by less than 20%, although most of the inactive drugs were measured only once. Data were analyzed by logarithm-probit plots.

um rapidly at 37°. With either filtration or microfuge assays, specific receptor binding is the same at 5, 10, and 30 min. At 0° using filtration the rate of association is much slower, requiring 25 min to attain half-maximal binding and about 2 hr to reach equilibrium.

Influence of Catecholamines. In calf striatal membranes, dopamine and apomorphine compete for [^{3}H]dopamine binding sites with similar potencies. Apomorphine inhibits [^{3}H]dopamine binding 50% at 4 nM concentration while the concentration for inhibition of binding by 50% (IC_{50}) for dopamine itself is 8 nM (Fig. 2). The slopes for inhibition of binding by apomorphine, dopamine, and norepinephrine are approximately parallel, with similar degrees of maximal inhibition. Epinine, the *N*-methylated form of dopamine, is about ⅕th as potent as dopamine, while the other catecholamines examined are much weaker. (−)-Norepinephrine is less than 1/20th as potent as dopamine with an (IC_{50}) of 170 nM. As has been shown for the dopamine-sensitive adenylate cyclase, the effects of norepinephrine and epinephrine are stereoselective, (−)-norepinephrine and (−)-epinephrine being about five times as potent as (+)-norepinephrine and (+)-epinephrine, respectively. Isoproterenol, the catecholamine with greatest affinity for β-norepinephrine receptors, is weaker than dopamine in competing for [^{3}H]dopamine binding sites by a factor of about 8000. *p*-Tyramine, which differs from dopamine only in lacking one ring hydroxyl substituent, has less than 0.1% the binding affinity of dopamine itself. The (+)- and (−)-isomers of amphetamine, a phenylethylamine structure without any hydroxyl groups, fail to influence dopamine binding at 100 μM concentration. The catechol amino acid, α-methyldopa, has no influence on [^{3}H]dopamine even in concentrations as high as 10,000 nM. Serotonin, which can compete with dopamine for nerve terminal uptake sites, is 1500-fold less potent than dopamine in inhibiting [^{3}H]dopamine binding.

To further ensure the specificity of dopamine binding sites, a large number of drugs with no known influence upon dopamine receptors pharmacologically were evaluated. None of these at 10 μM concentration interferes with [^{3}H]dopamine binding in calf striatal membranes (Table 1).

Effects of Phenothiazines and Related Drugs on Calf [^{3}H]Dopamine Binding Sites. Phenothiazines and other antischizophrenic drugs are thought to exert their clinical actions by blocking dopamine receptors associated with a dopamine-sensitive adenylate cyclase (2–5). Geometrical and optical isomers of certain of these drugs differ dramatically in their apparent ability to block dopamine receptors in pharmacological screening tests *in vivo* (8–10), and in their effects on the dopamine-sensitive adenylate cyclase (4, 11) and dopamine release from brain slices (12). (+)-Butaclamol, an effective antischizophrenic agent related to the phenothiazines, inhibits [^{3}H]dopamine binding to calf striatal membranes 50% at 125 nM (Fig. 3; Table 2). Butaclamol effects are stereospecific, since the (−)-isomer is weaker than the (+)-isomer by a factor of 135 in the calf. The geometrical isomers of the potent antischizophrenic thioxanthene, flupenthixol, differ in their potencies in competing for dopamine binding sites in calf striatal membranes by a factor of 50. The clinically active geometrical *cis* isomer of thiothixene is also about 300 times more potent in inhibiting [^{3}H]dopamine binding than the behaviorally inactive *trans* isomer (Fig. 4).

Fluphenazine, which is active in considerably lower doses than chlorpromazine, is one of the most potent of the clinically used phenothiazines, while promethazine is a phenothiazine with little or no efficacy in treating schizophrenia (13). In the calf corpus striatum, fluphenazine, chlorpromazine, and promethazine have IC_{50} values for inhibiting dopamine binding of 325 nM, 1500 nM, and 15,000 nM, respectively. Clozapine, a dibenzodiazepine with pharmacologic properties resembling the phenothiazines, is about 30% weaker than chlorpromazine in competing for calf dopamine sites. Imipramine, a tricyclic antidepressant closely related in structure to the phenothiazines but lacking antischizophrenic activity, requires concentrations of 25,000

Table 2. Comparison of [^{3}H]dopamine binding to rat and calf striatal membranes with rat dopamine-sensitive adenylate cyclase

Drug	IC_{50} (nM)			
			Rat adenylate cyclase	
	Calf binding	Rat binding	from ref. 4	from ref. 3
Dopamine	10	380	2,000	5,000
Apomorphine	5	180	2,000	
Epinine	35	180	1,500	
2-Amino-6,7-dihydroxy-(1,2,3,4)-tetrahydro-naphthalene	10		4,000	
(+)-Butaclamol	270	290	8.8	
α-Flupenthixol	270	210	1	
Fluphenazine	520	100	4.3	8
Chlorpromazine	1,500	250	48	66
Promazine	7,900	1,650	2,800	39
Promethazine	20,000	20,000	5,000	1,670
Haloperidol	900	4,800		220

The data shown in the first two columns are based on experiments in which the standard assay conditions were modified by addition of the following ions: NaCl, 120 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1 mM. In other experiments these ions were shown to have no effect on binding and drug effects. In the second column fresh rat corpus striatum was used. With the rat tissue, specific binding represented only about 21% of the total, whereas it was 47% of the total in the calf. For both tissues, blank values were taken as the mean of binding observed with 10^{-5} M dopamine, 10^{-5} M apomorphine, and 10^{-5} M (+)-butaclamol. IC_{50} values were determined by logarithm-probit analysis with four concentrations of each drug in triplicate.

nM to inhibit dopamine binding in calf striatal membranes by 50%.

The butyrophenones have pharmacological properties in animals and man which are essentially the same as those of the phenothiazines. The butyrophenones are, in general, more potent *in vivo* than the phenothiazines in eliciting apparent dopamine receptor blockade (14). However, they are relatively weak inhibitors of the dopamine-sensitive adenylate cyclase, and their influences on this enzyme do not correlate with their pharmacological potency *in vivo* (3–5).

Similarly, the butyrophenones, haloperidol, benperidol, and droperidol, are fairly weak inhibitors of dopamine binding in calf striatal membranes with IC_{50} values between 0.9 and 4.0 μM. Likewise, pimozide, a benzimidazolone, with pharmacological properties similar to those of the butyrophenones and which is highly potent as an *in vivo* blocker of dopamine receptors, is both a weak inhibitor of the dopamine-sensitive adenylate cyclase and of dopamine binding, requiring a concentration of 2.5 μM to inhibit dopamine binding to calf striatal membranes by 50%.

Comparative Properties of Calf and Rat [^{3}H]Dopamine Binding and Rat Dopamine-Sensitive Adenylate Cyclase Activity. The affinities of catecholamines for [^{3}H]dopamine binding sites in the rat are considerably greater than their potencies in enhancing the dopamine-sensitive adenylate cyclase (Table 2). Dopamine, epinine, and apomorphine are 10–20% as potent in enhancing dopamine-sensitive adenylate cyclase as in competing for [^{3}H]dopamine binding. By contrast, potent dopamine antagonists such as (+)-butaclamol, α-flupenthixol, and fluphenazine are 20 to 200 times more potent as inhibitors of the adenylate cyclase than of [^{3}H]dopamine binding in the rat.

Dopamine and apomorphine appear to have less affinity for the dopamine binding sites in striatal membranes of rat than calf. The IC_{50} values for dopamine and apomorphine in rat striatal membranes are 380 and 180 nM, respectively, compared to 10 and 5 mM for dopamine and apomorphine, respectively, in the calf. Isoproterenol is essentially inactive in both the rat and calf. Whereas (+)-butaclamol and α-flupenthixol have similar potencies in both rat and calf, haloperidol is 5-fold less potent in the rat than calf and all the phenothiazines are between 5- and 10-fold more potent in the rat than calf.

Evidence Indicating that Dopamine Binding Sites Do Not Involve Presynaptic Membranes. To examine whether dopamine binds to presynaptic dopamine terminals, unilateral lesions of the nigrostriatal dopamine pathway were performed by administration of 6-hydroxydopamine into the substantia nigra. Three weeks after lesions that destroy 90% of dopamine nerve terminals in the corpus striatum, we detect no difference in [^{3}H]dopamine binding between striatal preparations with and without lesions. Moreover, benztropine, one of the most potent known inhibitors of presynaptic [^{3}H]dopamine uptake, has very little affinity for [^{3}H]dopamine receptor binding sites in striatal membranes, with an IC_{50} value of 19 μM in the calf.

DISCUSSION

Specific [^{3}H]dopamine binding appears to involve postsynaptic dopamine receptors because of the close parallel between pharmacological and binding potencies of catecholamines and neuroleptic drugs. Relative affinities of ligands differ from [^{3}H]dopamine binding reported in an abstract by Seeman *et al.* (15). Recently we have also shown that [^{3}H]dopamine binding varies regionally like endogenous dopamine with highest levels in corpus striatum, olfactory tubercle, and nucleus accumbens and negligible amounts elsewhere (16).

Dopamine and other catecholamines have a greater affinity for binding sites than the adenylate cyclase, and the reverse holds for neuroleptic drugs. These discrepancies are maintained even when cyclase and binding are assayed under identical conditions (Creese, Burt, and Snyder, in preparation). The discrepancy appears to stem from the selective labeling by [^{3}H]dopamine of an "agonist" state of the dopamine receptor for which agonists have greater and antagonists lesser affinity. [^{3}H]Haloperidol labels the "antagonist" state of the dopamine receptor selectively (16). Butyrophenones and phenothiazines display about 100 times more and catecholamines 50 times less affinity for [^{3}H]haloperidol than [^{3}H]dopamine binding sites (16). Butyrophenones, whose very potent neuroleptic actions do not correlate with their affinities for the dopamine-sensitive adenylate cyclase or [^{3}H]dopamine binding, display high affinities (0.1–10 nM IC_{50} values) for haloperidol sites which correlate closely with pharmacological actions (Burt, Creese, and Snyder, in preparation).

We thank Janet Ryan for competent technical assistance and gratefully acknowledge the generous gifts of drugs by Drs. Phillip Seeman and Leslie Iversen. This research was supported by NIH Grants NS-10654 (to D.R.B.), MH-01598 (to S.J.E.), DA-00266, and MH-18501.

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